Trends in Parasitology

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Review

How Do Virus–Mosquito Interactions Lead to Viral Emergence?

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Arboviruses such as West Nile, Zika, chikungunya, dengue, and yellow fever viruses have become highly significant global pathogens through unexpected, explosive outbreaks. While the rapid progression and frequency of recent arbovirus outbreaks is associated with long-term changes in human behavior (globalization, urbanization, climate change), there are direct mosquito–virus interactions which drive shifts in host range and alter virus transmission. This review summarizes how virus–mosquito interactions are critical for these viruses to become global pathogens at molecular, physiological, evolutionary, and epidemiological scales. Integrated proactive approaches are required in order to effectively manage the emergence of mosquito-borne arboviruses, which appears likely to continue into the indefinite future.

Highlights

Arboviruses such as West Nile, Zika, chikungunya, dengue, and yellow fever viruses have become highly significant global pathogens through unexpected, explosive outbreaks.

Recent advances in next-generation sequencing have allowed rapid detection and identification of viruses, as well as tracing virus movement using phylogenetic analysis.

Evolutionary and modeling approaches, combined with experiments and new surveillance modalities, are attempting to predict virus emergence.

Vector competence and virus evolution studies – using selected mosquito species and neglected arboviruses – are helping to identify which virus may cause the next outbreak.

Integrated proactive approaches are required in order to effectively manage the emergence of mosquito-borne arboviruses, which appears likely to continue into the indefinite future.

Mosquito-Borne Viruses as the New Global Pathogens

Viral pathogens are major causes of morbidity and mortality among humans and animals. Efficient transmission of viruses between susceptible hosts is required in order for these agents to persist in nature and ultimately cause disease. Several mechanisms for this exist, and include direct contact, aerosol and sexual transmission, among others. A subset of viruses, termed arthropod-borne viruses (arboviruses) requires hematophagous arthropods, mainly mosquitoes and ticks, for transmission between vertebrates. In general, perpetuation of arboviruses requires vertebrate viremia so that arthropods acquire infectious virus along with nutrient-containing blood during feeding. Transmission of virus to a new host by an arthropod infected in this manner requires that this arthropod be a competent vector. In public health entomology, the term 'vector competence' refers to the inherent ability of a particular arthropod to transmit a particular virus. In competent vectors, virus is acquired during feeding, undergoes replication in gut tissue, disseminates to secondary sites of replication, including the salivary glands and is ultimately released into the arthropod's salivary secretions, where it may be inoculated into the skin and cutaneous vasculature of the host during subsequent feeding (Figure 1). Arboviruses, therefore, are those viruses that have evolved an intimate association with both a vertebrate and arthropod host in order to perpetuate in nature.

Mosquitoes are the most important vectors of arboviruses [1], although many are maintained by ticks [2], phlebotomines [3], and other arthropods [4]. The global health burden of mosquito-borne viruses is immense. It is commonly estimated that between 50 and 100 million cases of infection by dengue virus (DENV) serotypes 1–4 occur per year. Recent estimates placed the burden of DENV at 1.14 million disability-adjusted life-years in 2013 [5]. Most of the individuals who are at risk of DENV infection are at risk of other arboviruses, including yellow fever virus (YFV), chikungunya virus (CHIKV), and Zika virus (ZIKV) which share the same mosquito vector, *Aedes aegypti* [6]. Additional arboviruses that burden the health of individuals living in, or traveling to, the tropics include Japanese encephalitis virus (JEV), Venezuelan equine encephalitis virus (VEEV), Mayaro virus (MAYV), o'nyong nyong

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virus (ONNV), and many others. Temperate regions also experience seasonal epidemics of arboviral disease caused by West Nile virus (WNV), La Crosse virus (LACV), eastern equine encephalitis virus (EEEV), Jamestown Canyon virus (JCV) and related viruses. Although quantitative estimates of the collective burden of mosquito-borne arboviruses on human health worldwide do not currently exist, it is clear that their burden is enormous, and increasing [5,7,8].

The geographic distribution of many arboviruses has expanded in recent decades [6,9], resulting in infection of naïve populations and providing opportunities for new host–virus relationships to develop. For example, after incursions into Europe in the 1990s, WNV (genus *Flavivirus*) was introduced into the Americas in 1999 and rapidly spread from a small focus near New York City throughout the New World. Similarly, CHIKV (genus *Alphavirus*) spread from an African focus into Asia during the mid-2000s and was introduced into the Caribbean region in 2013 [10,11]. CHIKV is now endemic in the Americas and has caused over one million infections, many of which result in debilitating arthralgia [10]. ZIKV (genus *Flavivirus*) has also emerged in recent years [12]. Following an expansion from an African focus into the Pacific islands, the virus was introduced into South America and has spread throughout most of the range of its *Ae. aegypti* vector [8]. ZIKV has caused notable disease among developing fetuses and unexpected neurological disease among adults [13]. WNV, CHIKV, and ZIKV, along with DENV and YFV, underscore the emergence of mosquito-borne viruses as truly global pathogens. The combination of increased travel and trade has resulted in frequent exchange of pathogens and vectors across continents, such that the notion of 'geographic diseases' is increasingly irrelevant. Coupled with the rapid growth of tropical megacities, these exchanges continue to result in explosive epidemics of pathogens transmitted by mosquito vectors that require the human footprint on the environment in order to survive – vectors such as *Ae. aegypti* and *Culex quinquefasciatus*. The ongoing emergence of mosquito-borne viruses is occurring on a scale (geographic, economic, and human) that is without precedent in human history [7,8,14].

How, then, do mosquito–virus relationships lead to the emergence of these global pathogens (Figure 2, Key Figure)? This review examines the ways that mosquitoes influence the emergence of mosquito-borne viruses in order to provide perspectives on the history and future of this phenomenon. The first section examines two central concepts in public health entomology – vector competence and vectorial capacity – which outline basic mosquito–virus interactions and are key in understanding how mosquitoes impact virus emergence. These concepts are also required for readers to develop a basic understanding of the biology and epidemiology of arboviruses. The second section deals with new knowledge of the evolutionary relationships between viruses and their arthropod hosts. This section illuminates the complexities of arthropod–host interactions and how these can influence virus population biology and phenotype, sometimes leading to emergence. The third section provides a historical perspective on how mosquitoes influence arbovirus emergence by examining the cases of WNV, CHIKV, and ZIKV. Finally, we provide perspectives on the future emergence of mosquito-borne viruses, highlighting emerging mosquito-borne viruses that have yet to capture the attention of the general population.

Understanding Vector Competence and Vectorial Capacity

Arbovirus emergence is driven by the hematophagous behavior of their arthropod vectors. This unique mode of transmission has important consequences for the ecology of arboviruses. There are two concepts that are central to our understanding of arbovirus transmission and epidemiology: **vector competence** (see Glossary) and **vectorial capacity**.

Glossary

Antigenemia: describes levels of antigen in the blood. In the particular case of increased NS1 antigenemia mentioned here, it means that the more recent isolates of ZIKV result in increased NS1 levels in the blood of infected individuals.

E1 and E2 glycoproteins: structural proteins incorporated into the envelope of the alphaviruses, such as CHIKV. E1 and E2 are important for attachment, entry, and fusion events of the virus.

Enzootic transmission cycle: describes the natural transmission cycle of a pathogen between wild animals. The term 'enzootic' is equivalent to the term 'endemic' as used for human diseases.

Epizootic transmission cycle: refers to disease outbreaks among animal populations. The term 'epizootic' is equivalent to the term 'epidemic' as used for human diseases.

Vector competence: describes the ability of a particular arthropod to transmit a specific pathogen.

Vectorial capacity: describes the basic reproductive rate of a vector-borne pathogen by a particular vector species/population.

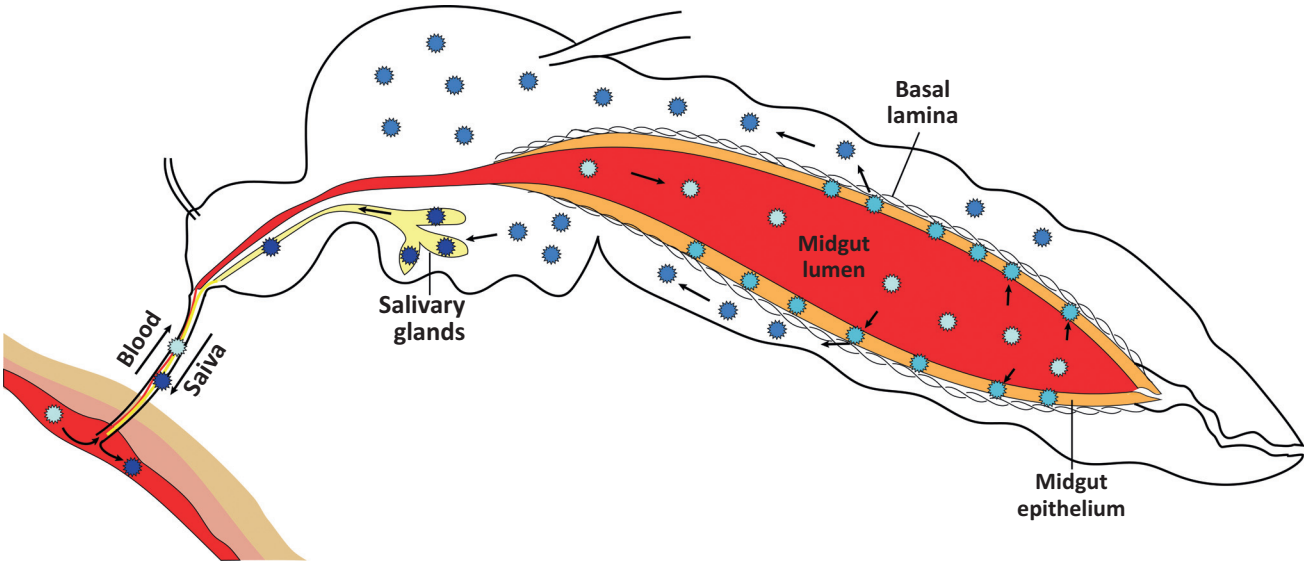

Figure 1. Different Tissue Barriers Determine Vector Competence in Mosquitoes. An arbovirus is taken up by the mosquito during an infectious blood meal. The virus infects the midgut epithelium and replicates before it passes the basal lamina into the hemolymph and disseminates throughout the mosquito's body. In order to be transmitted to the next host, the virus has to infect the salivary glands from where it can be released into the saliva and transmitted to the next host. Virus population genetic diversity is reduced stochastically as viruses pass through anatomical barriers to transmission, such as midgut infection and escape barriers, and salivary gland infection and escape barriers. Potential changes in virus populations that have passed through such bottlenecks are depicted as a change in color (increasingly dark blue). Through this genetic drift, as well as positive selection in the mosquito, new genotypes may emerge.

As described above, vector competence defines the ability of a particular arthropod to transmit a given virus. Vector competence has been studied extensively in mosquitoes, and it is determined by both genetic and nongenetic (e.g., environmental) factors. It may vary depending on mosquito species, local mosquito populations, and even individual mosquitoes. Importantly, vector competence is a quantitative rather than a qualitative quantity: rarely do all mosquitoes of a given species or population transmit a given virus. Thus, vector competence is usually expressed as a proportion (e.g., 34% of *Culex tarsalis* mosquitoes transmit WNV after 10 days incubation). Vector competence of a specific mosquito population is also dependent on the virus, and even different isolates of the same virus species may result in changes in vector competence of a mosquito population [15,16]. Generally, there are four major barriers that the virus must cross within the mosquito in order to be transmitted (reviewed in [17]). First, when a mosquito ingests an infectious bloodmeal, the virus must successfully infect and replicate in the midgut epithelial cells (Figure 1). The midgut is the first tissue in which virus–mosquito cell interactions occur that can shape the outcome of infection. Mosquitoes in which the virus cannot establish an infection have a midgut infection barrier (MIB). This can occur due to genetic factors, such as lacking expression of receptors on the cell surface, or nongenetic determinants such as microbiome density and composition [18–20]. However, once a virus has established midgut infection it must cross the basal lamina surrounding the midgut epithelium in order to disseminate throughout the rest of the mosquito (Figure 1). When virus replication is limited to the midgut and dissemination does not occur, mosquitoes are said to have a strong midgut escape barrier (MEB). The basal lamina of the midgut presents a physical barrier for the virus, and the thickness of the basal lamina has previously been linked to decreased dissemination of DENV-1 in different *Aedes albopictus* populations [21]. Furthermore, it has been shown that smaller, nutritionally deprived *Aedes triseriatus* mosquitoes have increased LACV

Key Figure

Summary of Factors Influencing Arbovirus Emergence

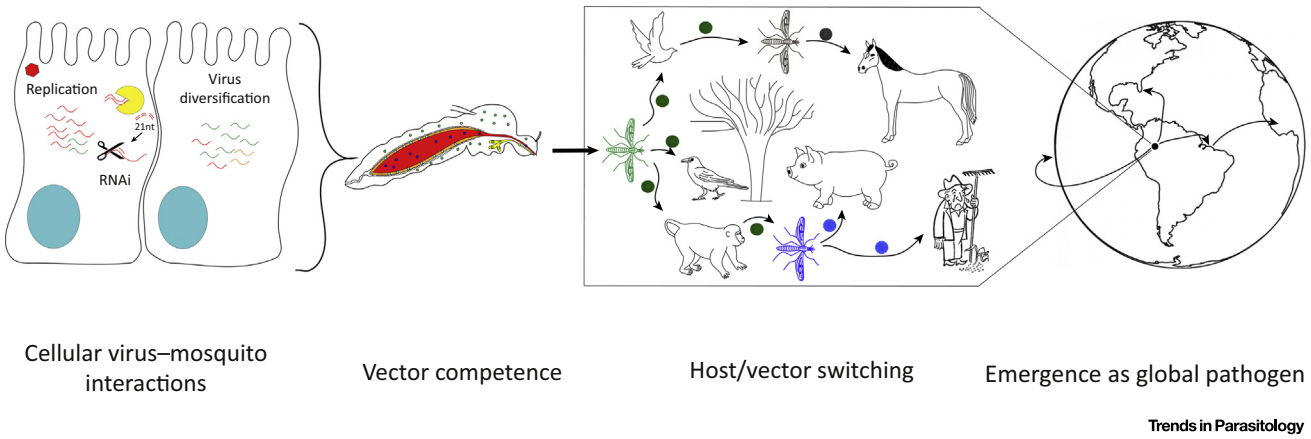

Figure 2. Cellular and molecular interactions, such as RNAi, drive virus diversification in the mosquito. Differences in mosquito vector competence, and bottlenecks that the virus encounters during dissemination within a mosquito, can result in further divergence of the virus population and drive virus evolution and emergence. Due to urbanization and deforestation, humans and livestock are frequently in close proximity to mosquito vectors and to vertebrate hosts maintaining viruses in sylvatic/enzootic transmission cycles. Human settlements may also bring along anthropophagic mosquitoes such as *Aedes aegypti* and *Aedes albopictus*, which may encounter viremic reservoir hosts, such as primates. Interactions between new vector mosquitoes and vertebrate hosts can drive arbovirus evolution and emergence. Due to intense travel, and ubiquitous distribution of *Aedes* spp. mosquitoes, such spillover events may easily lead to the outbreak of a new global pathogen.

dissemination rates compared to larger mosquitoes reared on a normal or rich larval diet [22,23], which may also correlate with the development of a thick basal lamina. However, it has recently been shown that the uptake of a bloodmeal alters expression of specific enzymes in the mosquito midgut, including collagenases, which results in transient degradation and increased permissibility of the basal lamina, allowing CHIKV to disseminate [24,25]. Viruses with slower replication rates, such as DENV, may not benefit as much from early transient degradation of the basal lamina following a bloodmeal. Another possible midgut escape route for arboviruses may be via tracheal or neuronal cells. Dong and colleagues [24] recently showed that CHIKV can infect tracheal cells connected to *Ae. aegypti* midguts. When the virus has disseminated from the midgut it replicates in other mosquito tissues, including the fat body, hemocytes, nerve tissue, and muscle tissue (depending on the virus) ultimately reaching the salivary glands, the next crucial anatomical barrier to infection, the 'salivary gland infection barrier' (SGIB). Upon salivary gland infection, the virus replicates and is deposited in the apical cavities of acinar cells in order to be expectorated with saliva (Figure 1). However, not all mosquitoes will be able to expectorate virus (for reasons yet unknown) and thus have a 'salivary gland escape barrier' (SGEB).

Other important mechanisms to limit virus replication throughout the mosquito body are mosquito antiviral immune responses (reviewed in [26,27]). The most specific and potent mosquito antiviral defense is RNA interference (RNAi). During infection of a mosquito cell, viral dsRNA intermediates are recognized by the endonuclease Dicer2 and cleaved into 21 nt virus-derived small RNAs. These small RNAs are integrated into the RNA-induced silencing complex (RISC) and can target viral RNA for degradation. This sequence-specific response can be very efficient at controlling viral replication, and many viruses have evolved mechanisms to

antagonize or evade the RNAi response [28]. All mosquito antiviral responses may pose selective pressures on the virus, but RNAi is unique in its sequence specificity, which poses a direct evolutionary pressure on the viral genome. Mosquito barriers and antiviral responses together contribute to the overall phenotype of vector competence.

Vectorial capacity is a second critical concept that describes the basic reproductive rate of a vector-borne pathogen by a particular vector species/population (Figure 3), and highlights the power of mosquitoes as drivers of virus emergence. Factors which influence vectorial capacity are vector density with respect to host (m), the daily probability of the host being fed upon (a), vector competence (VC), the probability of daily survival (*P*) and the extrinsic incubation period (n). The factors that influence vectorial capacity, and lead to arbovirus emergence, have been reviewed and discussed extensively [29,30]. Briefly, the most influential variables in the formula are the probability of daily mosquito survival and the extrinsic incubation period (EIP). The EIP refers to the amount of time that it takes for a virus to infect the midgut and disseminate to the saliva (i.e., the time between uptake of virus and ability to transmit). If the probability of mosquito daily survival is low and the EIP long, the likelihood of transmission is low. Extensive mosquito-control programs that shorten the lifespan can thus efficiently reduce transmission. However, the EIP is affected by environmental conditions, such as temperature [31–33], as well as genetic factors [34–36] influencing vector competence of the mosquito population. Viruses may adapt to faster dissemination in susceptible mosquito species and thus shorten the EIP [37].

Another important variable is the probability of a particular host being fed upon, or the degree of host focus. This variable, 'a' in the vectorial capacity equation, is a proportion (e.g., 80%, or 0.8 of mosquito X bloodmeals are taken from host Y) and is raised to the second power to reflect the need for susceptible hosts to be bitten twice in order for perpetuation to occur. Different mosquito species vary in their blood-feeding behavior and host preference. The mosquito species *Ae. aegypti*, for example, has adapted to life around humans, in particular urban areas of the tropics and subtropics, and mosquitoes of this species feed almost exclusively on human

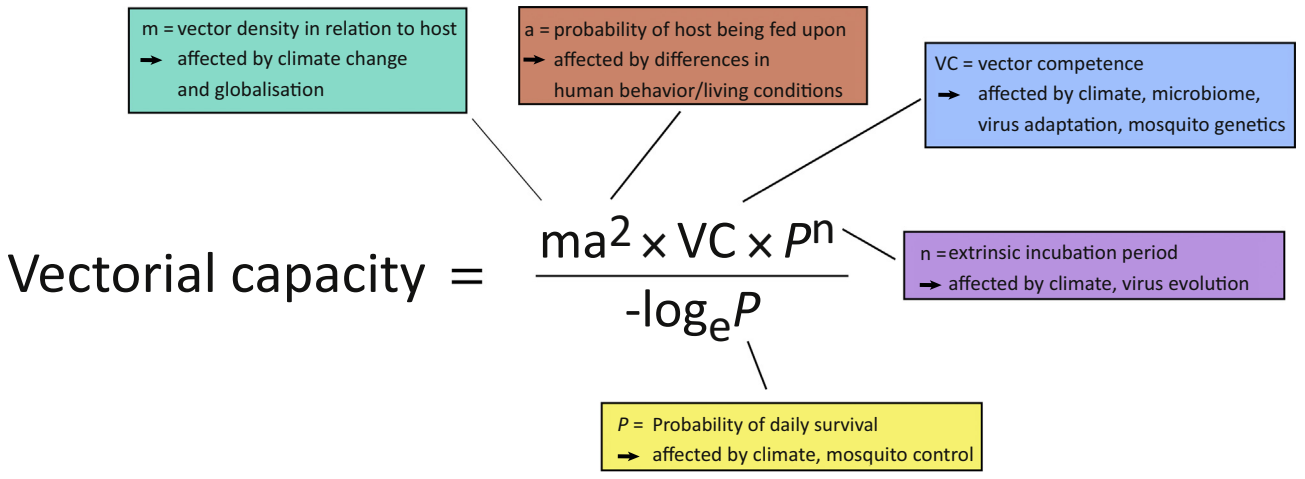
m = vector density in relation to host
affected by climate change and globalisation
a = probability of host being fed upon
affected by differences in human behavior/living conditions
VC = vector competence
affected by climate, microbiome, virus adaptation, mosquito genetics
Vectorial capacity = ma² x VC x Pⁿ / -log_e P
n = extrinsic incubation period
affected by climate, virus evolution
P = Probability of daily survival
affected by climate, mosquito control
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Figure 3. Vectorial Capacity. The vectorial capacity formula describes the total number of future infectious bites arising from mosquitoes biting an individual infectious host on a single day. It consists of five factors: vector density with respect to host (m), the daily probability of the host being fed upon (a), vector competence of the mosquito population (VC), the probability of daily survival (*P*) and the extrinsic incubation period (n). None of these factors are constants, but variables which depend on both environmental influences as well as specific virus–mosquito interactions as indicated.

hosts and frequently feed indoors [38]. This behavior makes them extremely efficient vectors for viruses such as DENV, CHIKV, and ZIKV, which replicate to high titers in human hosts. In contrast, *Ae. albopictus*, which can also serve as a vector for DENV, CHIKV, and ZIKV, is more likely to feed outside, and while it is still anthropophilic, it is more of an opportunistic feeder [39]. Other mosquitoes, such as several species within the genus *Culex,* may feed on a human host, but tend to prefer birds [40]. This makes them efficient vectors for WNV and other zoonotic arboviruses. WNV infection of human or horse hosts is incidental and results in a 'dead end' for the virus due to inefficient replication and consequent low viremia. Thus, the competence of a given mosquito vector for an arbovirus may be irrelevant if that mosquito feeds only infrequently on susceptible vertebrate hosts.

Virus adaptation to new mosquito vectors clearly shapes the patterns and dynamics of arbovirus emergence. Generally, viruses with an *Ae. aegypti*-driven urban life cycle emerged from enzootic progenitors that circulated between nonhuman primates and sylvatic mosquito species such as *Aedes africanus* or *Aedes furcifer* [8,41]. Adaptation to transmission by *Ae. aegypti* mosquitoes provided access to a new and abundant vertebrate host, humans. This has resulted in explosive outbreaks as seen before for ZIKV, which originated in a sylvatic cycle in Africa. Similarly, there are likely numerous viruses in current sylvatic viruses that may cause disease outbreaks in the future, some of which we already know, many of which we may be unaware of.

Mosquitoes as Sources of Virus Genetic Diversity

Arbovirus transmission requires active virus replication both in the arthropod vector and the vertebrate host. Arboviruses are thus subject to selective pressures from two evolutionarily distant hosts. In vertebrates, where purifying selection is strong [42,43], these pressures include innate and adaptive immune responses. Mosquitoes lack a classic adaptive immune system and do not produce interferon. However, Jak/STAT signaling and other signaling pathways, such as Toll, have been identified as antiviral responses against DENV [44,45], WNV [46], Semliki Forest virus [47], and ONNV [18]. RNAi is the main antiviral defense in mosquitoes [27], which has direct consequences for virus intrahost evolution in mosquitoes (which can significantly impact virus emergence). Virus-derived small RNAs inhibit virus replication and translation by directly binding to complementary viral RNA [27]. The generation and perpetuation of novel sequences of viral RNA (i.e., containing mutations) may thus be beneficial for the virus within the mosquito because small RNAs will not match with perfect complementarity. Experimental evolution studies have confirmed the hypothesis that RNAi targeting by the arthropod vector leads to diversification of arbovirus genomes [48–50]. Rapid evolution and generation of a complex virus population could be particularly beneficial for the virus in order to reduce complementary binding, and because small RNAs have been shown to spread from cell to cell and may thus prepare neighboring cells for imminent virus infection [51]. However, these rapidly generated complex viral populations encounter bottlenecks within the mosquito when crossing the MEB, the SGIB, and during transmission of saliva itself [52,53]. These bottlenecks vary in their size between different mosquito species and most likely virus–vector combinations. In *Cx. quinquefasciatus*, for example, WNV diversification is high, purifying selection is relatively weak, and bottlenecks are significant, resulting in high divergence of WNV populations in the saliva compared to the virus contained in the blood-meal [52]. Different mosquito species may thus alter the virus population that is ultimately transmitted. Additionally, it has been shown that *Culex* mosquitoes may transmit unique WNV populations during each feeding episode [54], which is a further example of how mosquito infection promotes virus diversification. However, it is not known if the same phenomenon occurs with other viruses and mosquito species.

Rapid intrahost evolution of a mosquito-borne virus in the mosquito may result in the emergence of a new variant with a replication advantage in the vertebrate host or a new tissue tropism resulting in increased disease severity. For instance, VEEV emerges frequently from **enzootic** to **epizootic** transmission cycles, with horses serving as amplifying hosts causing large outbreaks in both humans and horses. These epizootic strains appear to have only a few amino acid changes in the **envelope glycoprotein E2** in common compared to the enzootic strains [55]. There are many VEEV strains currently being transmitted in enzootic cycles, posing a constant threat in South and Central America [56].

While virus adaptation to replication in mosquitoes may reduce the ability of the virus to replicate to high titers in the mammalian host, such adaptations can also affect pathogenesis in undesirable ways. This can be seen in EEEV, for example, which does not reach high viremia in humans, but can cause severe to fatal encephalitis. It has recently been shown that the virus cannot replicate in myeloid cells of mice due to a miRNA target site in the 3′UTR for miRNA 142-3p, a myeloid cell-specific miRNA [57]. However, while mutating this target sequence led to an increase in viremia and replication in peripheral tissues in mice, it resulted in a decrease in replication in mosquito cells and in infectivity of mosquitoes, suggesting that this sequence is important for replication in mosquitoes. This is one example of how adaptation to the vector may interfere with replication in a mammalian host.

Conversely, insect-specific viruses may have at some point developed mechanisms to inhibit mosquito antiviral defenses, which may also help the virus replicate efficiently in mammalian cells. One such mechanism is the generation of subgenomic flaviviral RNA (sfRNA) during replication. Flaviviruses have complex secondary structures in the 3′UTR, which stall the 3′→5′ exonuclease XRN1, resulting in the generation of sfRNAs. These sfRNAs inhibit mRNA degradation by sequestering XRN1, both in mosquito and mammalian cells. sfRNAs also suppress interferon signaling in vertebrate cells and are important for cytopathicity as well as pathogenicity in mice [58]. Production of sfRNAs is also important for replication and dissemination of flaviviruses in mosquitoes [59,60], which may be due to both repression of XRN1 and their other function as suppressors of the mosquito RNAi machinery [61,62]. However, it has recently been shown that DENV sfRNA patterns differ when virus is passaged in mosquito cells compared to mammalian cells [63], suggesting that while sfRNA generation is important in both cell types it is not optimized for one over the other. Overall, sfRNAs may have evolved in mosquitoes (similar yet less complex 3′UTR structures exist in insect-specific flaviviruses), but have an important role in vertebrate cells that is related to, but distinct from, its role in mosquitoes.

Virus–Mosquito Interactions Have Previously Shaped Virus Emergence

In recent decades, WNV, CHIKV, and ZIKV have emerged as global pathogens in explosive outbreaks that have caused significant human morbidity and mortality. The general phenomenon of formerly geographically restricted arboviruses emerging on a global scale has become common enough, and it is perhaps best considered a new *status quo*. Several factors that have led to this phenomenon have been extensively discussed, including in this review. These include global increases in travel and trade, the rise of tropical megacities, and the decline of public health programs to manage vector mosquitoes [9,64,65]. Further, as we have seen above, arboviruses have the capacity for explosive outbreaks due to their coadaptation to mosquitoes, which carry these agents between individuals with direction (i.e., as a vector). However, in each case (WNV, CHIKV, and ZIKV) the virus adapted to local conditions during global spread, which maximized transmission potential (and health burden). These changes highlight the ways that the association of viruses with mosquitoes can lead to their emergence.

WNV was introduced into the Americas in 1999, most likely from the Middle East [66]. Molecular epidemiologic studies confirmed that the virus remained fairly homogeneous until approximately 2001, when a new virus genotype emerged in central USA. During the next 2 years, this genotype (somewhat erroneously called the 'WN02' lineage because it was first recognized during 2002) outcompeted the introduced 'NY99' genotype to the extent that the NY99 genotype seems to have become extinct. The most notable difference between this genotype and the introduced genotype was a mutation that resulted in a conservative valine to alanine change on the exposed surface of the WNV envelope glycoprotein [67,68]. This mutation conferred faster transmission by *Culex pipiens* mosquitoes, important vectors of WNV in North America [37]. The reduced EIP of the WN02 genotype enhances vectorial capacity (see above) because mortality has less time to act on WN02-infected mosquitoes before they can transmit virus to a new host. This enhanced vectorial capacity of the WN02 genotype coincided with a massive WNV outbreak in North America in 2002. Thus, specific virus–mosquito interactions between WNV and *Cx. pipiens* led to epidemiological changes (via vectorial capacity) that contributed to a significant and, at the time, unprecedented arbovirus outbreak in the USA.

In contrast, CHIKV provides evidence of how an arbovirus can acquire the capacity for efficient transmission by a new vector mosquito species with disastrous consequences. The current global CHIKV epidemic began when the virus emerged in coastal Kenya around 2004. From there, it spread throughout the Indian Ocean region. During this process of emergence, the virus acquired a mutation to the coding sequence of the **envelope glycoprotein E1** that resulted in the substitution of a valine for an alanine at position 226 (A226V) of the protein [69]. This mutation rendered the virus more transmissible by the highly abundant *Ae. albopictus* mosquito [70] (i.e., increased the competence of *Ae. albopictus*). Since the competence of any given vector (again, see above) clearly impacts the reproductive rate of the virus, the acquisition of the A226V mutation conferred an advantage and spread rapidly [70]. Initial adaptation to *Ae. albopictus* later allowed for mutations in CHIKV E2 to develop, which further enhanced infection of the *Ae. albopictus* midgut [71]. Interestingly, the CHIKV that was introduced into the Caribbean in 2013 lacked the A226V mutation, and this mutation has not yet been detected in CHIKV from the Americas.

Most recently, ZIKV has emerged as a global pathogen in a manner similar to that of WNV and CHIKV before it. The consequences of ZIKV infection were not fully understood prior to this emergence, and are now notorious: microcephaly in developing fetuses and Guillain–Barré syndrome in some infected adults [13]. Further, it is now clear that ZIKV may be transmitted sexually [72,73]. Several mutations have been described in the literature that impact the ZIKV phenotype in potentially significant ways. A mutation that changes an alanine at position 188 of the ZIKV NS1 protein to a valine has been associated with enhanced infectivity to *Ae. aegypti* mosquitoes via increased NS1 **antigenemia** [74]. Similarly, the serine to asparagine mutation at amino acid 139 (S139N) of the prM protein, that appears to have arisen around 2013, has been shown to contribute to pathogenesis in developing fetuses [75]. It is not yet clear what specific interactions could have produced either of these changes and how they may impact other important ZIKV phenotypes. This promises to be a fruitful area for future research.

Future Arbovirus Threats

The recent history of mosquito-borne virus emergence, coupled with what we know about the molecular and ecological interactions that facilitate their transmission, indicate that new explosive outbreaks of arbovirus disease are likely to occur in the future. Several arboviruses (Table 1) currently circulate at low levels in geographically limited settings that result in few human cases. While all of these viruses have the (theoretical) capacity to spread rapidly and

Table 1. Selected Arboviruses with Currently Limited Geographic Distribution and Disease Incidence

Genus	Virus	Suspected mosquito vector	Geographic distribution	Disease
Alphavirus	Mayaro virus (MAYV)	*Haemagogus* spp.	South and Central America	Febrile illness, arthralgia, myalgia
	Una virus (UNAV)	*Psorophora* spp.	South America	Febrile illness, arthralgia, myalgia
	Pixuna virus (PIXV)	Unclear	South America	Febrile illness, encephalitis
	Rio Negro virus (RNV)	Unclear	South America	Febrile illness, myalgia
	Tonate virus (TONV)	Unknown	French Guiana	Febrile illness, encephalitis
	Everglades virus (EVEV)	*Culex cedecei*	Florida	Fever, headache, myalgia
	Mucambo virus (MUCV)	*Culex* spp.	South America	Febrile illness, arthralgia, myalgia
	Trocara virus (TROCV)	*Aedes serratus*	South America	Unknown
	O'nyong nyong virus (ONNV)	*Anopheles* spp.	Sub-Saharan Africa	Febrile illness, arthralgia, myalgia
Flavivirus	Spondweni virus (SPONV)	*Aedes circumluteolus*	Sub-Saharan Africa	Febrile illness, arthralgia, myalgia
	Dengue virus type 5 (DENV-5)	*Aedes* spp.	South East Asia	High fever, arthralgia, myalgia
	Rocio virus (ROCV)	*Aedes* spp., *Psorophora* spp.	South America	Febrile illness, encephalitis
Orthobunyavirus	Oropouche virus (OROV)	Mainly *Culicoides* biting midges[a]	South America	High fever, arthralgia, myalgia
Phlebovirus	Rift Valley fever virus (RVFV)	>40 species of mosquitoes	Sub-Saharan Africa	Febrile illness, arthralgia, myalgia

[a]Oropouche virus is possibly also transmitted by *Aedes* spp. and *Coquillettidia* spp. mosquitoes.

cause outbreaks, some of them seem more likely than others to cause large-scale epidemics in the near future.

One of these viruses is MAYV, which has been circulating in parts of South and Central America since at least 1954 when it was first isolated in Trinidad [76]. Disease symptoms are similar to those of CHIKV infection, including fever, rash, myalgia, and arthralgia. MAYV is suspected of being transmitted between nonhuman primates by vector mosquitoes of the genus *Haemagogus*; however, *Ae. aegypti* and *Ae. albopictus* are capable of transmission in an experimental setting [77,78]. While few human infections have been reported overall, a relatively large outbreak of 77 cases occurred in 2010 in Venezuela [79], and MAYV received some media attention due to a human case in Haiti in 2015 [80]. Historically, MAYV infections occurred predominantly in northern South America, in regions bordering the Amazon basin [81]. Since there was no history of travel in the Haitian case, infection probably occurred through local mosquito transmission. The patient was also coinfected with DENV-1, possibly suggesting that infection occurred through the bite of an *Ae. aegypti* mosquito. Since there are no nonhuman primates on Hispaniola, this may indicate the presence of a local human–mosquito transmission cycle. However, MAYV was previously isolated from a migrating bird in Colorado [82], suggesting another potential route of MAYV introduction into Haiti (and a source of infection of local mosquitoes). Whether continuous local transmission was established remains unknown. While reports of potential recent recombinant MAYV strains [83] are of some concern, recombination of alphaviruses is likely a rare event. The only recombinant alphaviruses described are the three members of the Western equine encephalitis virus serocomplex, which are recombinants of a strain of EEEV and a Sindbis-like virus [84]. However, with a multitude of alphaviruses circulating in South and Central America, and the recent addition of CHIKV, a small chance of recombination events remains. This could result in rapid emergence of new viruses with altered transmission phenotypes and pathogenesis. The risk of MAYV emergence has been discussed in detail in several recent reviews [85–87].

In addition, a little-known flavivirus of some concern is Spondweni virus (SPONV). SPONV is currently circulating solely in sub-Saharan Africa in sylvatic cycles involving zoophilic *Aedes* mosquitoes. Peri-domestic mosquitoes, such as *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*, do not appear to be competent vectors [88]. However, as previously discussed, adaptation to an urban transmission cycle may be achieved by only one or a few mutations in the virus genome that increase the infection potential for *Ae. aegypti* or *Ae. albopictus* mosquitoes. Current research on SPONV is limited, but recent evidence shows its potential to be sexually transmitted among mice, albeit inefficiently [89]. Our current lack of understanding of SPONV pathogenesis and transmission warrants further investigation. The zoonotic potential of arboviruses currently circulating in Africa has been reviewed elsewhere [90].

Concluding Remarks

Over recent decades, arboviruses have truly emerged as global pathogens. Viruses previously existing only in local transmission cycles in rural tropical settings are now distributed worldwide, causing devastating disease outbreaks. This is largely due to the now global distribution of mosquito species with high vector potential, such as *Ae. aegypti* and *Ae. albopictus*, as well as human travel and population density in tropical megacities. Virus–mosquito interactions and mosquito adaptation to humans contribute significantly to arboviral emergence. Future research should be aimed at increasing our understanding of neglected arboviruses, novel surveillance methods, and implementation of surveillance programs to recognize spillover events and arbovirus outbreaks early on (see Outstanding Questions). Surveillance for arboviruses and also invasive mosquito species can help with implementation of rapid vector-control responses. As with all arboviruses, vector control will be crucial for the prevention and containment of future arbovirus emergence. The development of simple inexpensive diagnostic tests for a large selection of pathogens may also help to identify and contain an outbreak. Moreover, the mosquito–virus interaction has facilitated the rapid emergence of several arboviruses, a pattern that is likely to continue, requiring an integrated approach to outbreak management.

Outstanding Questions

Can the emergence of mosquito-borne arboviruses be predicted and pre-emptively managed?

Which sylvatic/enzootic arboviruses can be maintained in a transmission cycle exclusively between humans and mosquitoes?

What vector-control mechanisms and other preventative measures can specifically help to prevent or contain future outbreaks?

What are the molecular mechanisms that underpin virus host shifts?

To what extent can evolutionary approaches contribute to predictive efforts?

Defining transmission and/or pathogenesis-altering mutations to a viral genome using experimental studies is, in some cases, highly tractable. However, frequently, these studies may result in so-called 'gain-of-function' variants. Are the benefits of understanding the plasticity of viral genotypes and phenotypes worth the risk of generating gain-of-function variants?

Is our society willing to invest in determining what arboviruses may emerge next?

Systems for monitoring arbovirus emergence exist, but are difficult to implement at an appropriate scale. Further, many modern systems for monitoring health 'events' such as arbovirus emergence rely on passive reporting and social media. Appropriate surveillance systems should focus on detecting agents that are early in the process of emergence and contain mechanisms to support critical research on an ongoing basis. Doing so requires financial commitment and political will.

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